

Atty. Dkt. No. 041673-2092

Listing of Claims

This listing of claims supercedes all prior listing of claims, and includes amended claims. Please amend claims 2, 3, 4, 12 and 37 as follows, and withdraw, without prejudice, claims 5, 6, 9, 10, 13, 14, 16-21, 23-26, and 42-67. Claims withdrawn are without prejudice to their later presentation and examination.

1. (Previously Cancelled).
2. (Presently Amended) A nucleic acid molecule encoding a chimeric TNFa ligand polypeptide, comprising a first polynucleotide encoding a Domain III fragment of CD154-a tumor necrosis factor ligand other than TNFa lacking a metalloproteinase cleavage site and a second polynucleotide encoding a Domain IV fragment of TNFa protein that binds to a TNFa receptor.
3. (Presently Amended) The nucleic acid molecule of claim 2 further comprising a third polynucleotide that encodes Domain II fragment of CD154 ~~the other tumor necrosis factor ligand~~.
4. (Presently Amended) The nucleic acid molecule of claims 2 or 3, further comprising a fourth polynucleotide that encodes a Domain I fragment of CD154 ~~the other tumor necrosis factor ligand~~.
5. (Withdrawn) The nucleic acid molecule of claims 2, 3 or 4 wherein the first polynucleotide further encodes a Domain IV fragment of the other tumor necrosis factor ligand.
6. (Withdrawn) The nucleic acid molecule of claim 2, wherein the other tumor necrosis factor ligand is selected from the group consisting of CD154, CD70, Fas ligand, NGF, CD30, TNF β , 4-1BBL and TRAIL.
7. (Previously Cancelled).
8. (Previously Amended) The nucleic acid molecule of claim 2, wherein the second polynucleotide encodes a Domain IV fragment of native TNFa that lacks a cleavage site of TNFa protein.
9. (Withdrawn) The nucleic acid molecule of claim 2, wherein the first polynucleotide further encodes Domain I, II or III fragments of a tumor necrosis factor ligand selected from the group consisting of CD154, CD70, Fas ligand, NGF, TNF β , CD30, 4-1BBL and TRAIL, and the second polynucleotide encodes Domain IV of TNFa protein.

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10. (Withdrawn) The nucleic acid molecule of claim 9 wherein the first polynucleotide further encodes Domain I, II or III fragments of CD154 protein.
11. (Previously Amended) The nucleic acid molecule of claim 2 further comprising a linker domain encoding a peptide of at least one amino acid that links the first polynucleotide to the second polynucleotide.
12. (Presently Amended) The nucleic acid molecule of claim 2, comprising a nucleotide sequence selected from the group consisting of SEQ.ID. NO. 1, ~~SEQ.ID. NO. 2, SEQ.ID. NO. 3 and SEQ.ID. NO. 4.~~
13. (Withdrawn) The nucleic acid molecule of claim 2, wherein the chimeric TNFa comprises an amino acid sequence selected from the group consisting of SEQ.ID. NO. 5, SEQ. ID. NO. 6, SEQ.ID. NO. 7 and SEQ.ID. NO. 8.
14. (Withdrawn) A chimeric TNFa, comprising a Domain III fragment of a tumor necrosis factor ligand other than TNFa lacking a matrix metalloproteinase cleavage site and a Domain IV fragment of TNFa that binds to a TNFa receptor.
15. (Previously Cancelled).
16. (Withdrawn) The chimeric TNFa of claim 14 that is less susceptible to cleavage from the surface of cells than native TNFa.
17. (Withdrawn) The chimeric TNFa of claim 16, wherein the cleavage rate of the chimeric TNFa is at least 90% less than that of native TNFa.
18. (Withdrawn) The chimeric TNFa of claim 14, further comprising a Domain II fragment of the other tumor necrosis factor ligand.
19. (Withdrawn) The chimeric TNFa of claims 14 or 18, further comprising a Domain I fragment of the other tumor necrosis factor ligand.
20. (Withdrawn) The chimeric TNFa of claims 14, 18 or 19, further comprising a fourth Domain IV fragment of the other tumor necrosis factor ligand.
21. (Withdrawn) The chimeric TNFa of claim 14, wherein the other tumor necrosis factor ligand is selected from the group consisting of CD154, CD70, Fas ligand, NGF, CD30, TNF β , 4-1BBL and TRAIL.

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22. (Previously Cancelled).
23. (Withdrawn) The chimeric TNFa of claim 14, wherein the Domain IV fragment lacks a cleavage site of TNFa protein.
24. (Withdrawn) The chimeric TNFa of claim 14, comprising domains I, II and III, of a tumor necrosis factor ligand selected from the group consisting of CD154, CD70, Fas ligand, NGF, CD30, TNF β , 4-1BBL and TRAIL, and domain IV of TNFa protein.
25. (Withdrawn) The chimeric TNFa of claim wherein one or more of the domains I, II and III are of CD154 protein.
26. (Withdrawn) The chimeric TNFa of claim 14, further comprising a linker domain encoding a peptide of at least one amino acid that links the Domain III fragment to the Domain IV fragment.
27. (Previously Amended) An expression vector, comprising the nucleic acid molecule of claim 2.
28. (Original) An expression vector, comprising the nucleic acid molecule of claim 3.
29. (Previously Amended) An expression vector, comprising the nucleic acid molecule of claim 4.
30. (Withdrawn) An expression vector, comprising the nucleic acid molecule of claim 9.
31. (Withdrawn) An expression vector, comprising the nucleic acid molecule of claim 11.
32. (Original) The expression vector of claim 27, further comprising viral DNA or bacterial DNA.
33. (Original) The expression vector of claim 32 wherein said viral DNA is selected from the group consisting of adenoviral DNA or retroviral DNA.
34. (Original) The expression vector of claim 32, wherein at least a portion of the vector comprises adenoviral DNA.
35. (Original) The expression vector of claim 27, further comprising a promoter region.
36. (Original) The expression vector of claim 27, further comprising a polyadenylation signal region.

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37. (Presently Amended) A genetic construct comprising the nucleic acid molecule according to claim 2 operatively linked to a promoter sequence and to a polyadenylation signal sequence.
38. (Original) A host cell, comprising an expression vector according to claim 27 or a genetic construct according to claim 37.
39. (Original) The host cell of claim 38, wherein the cell is a mammalian cell.
40. (Original) The host cell of claim 39, wherein the cell is a tumor cell.
41. (Original) The host cell of claim 39, wherein the cell is an antigen presenting cell.
42. (Withdrawn) A process for producing a chimeric TNFa of claim 14 comprising culturing a host cell of claim 38 under conditions suitable to effect expression of the protein.
43. (Withdrawn) A method for increasing the concentration of a ligand capable of binding to a TNFa receptor on the surface of a cell, comprising introducing into the cell a nucleic acid molecule encoding a chimeric TNFa polypeptide according to claim 2, whereby the chimeric TNFa polypeptide is less susceptible to cleavage from the surface of the cells than a TNFa protein.
44. (Withdrawn) The method of claim 43, wherein the comprises an expression vector according to claim 27 or a genetic construct according to claim 37.
45. (Withdrawn) The method of claim 44 wherein the cell is a mammalian cell.
46. (Withdrawn) The method of claim 44 wherein the cell expresses a TNFa receptor on its surface.
47. (Withdrawn) A method for inducing apoptosis of a cell expressing a TNFa receptor, comprising introducing into the cell an encoding a chimeric TNFa polypeptide according to claim 1 or claim 2 wherein the chimeric TNFa polypeptide is expressed on the surface of the cell.
48. (Withdrawn) A method for inducing activation of an immune system cell, comprising introducing into the cell a nucleic acid molecule encoding a chimeric TNFa polypeptide according to claim 2 wherein the chimeric TNFa polypeptide is expressed on the surface of the cell.
49. (Withdrawn) A method for treating neoplasia in a patient comprising introducing into a neoplastic cell a nucleic acid molecule encoding a chimeric TNFa polypeptide according to claim 2 wherein the chimeric TNFa polypeptide is expressed on the surface of the cell.

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50. (Withdrawn) The method of claim 49 further comprising: obtaining the neoplastic cell from a human patient; infusing the neoplastic cell back into the patient after having introduced into the cells the nucleic acid molecule encoding the chimeric TNFa polypeptide.
51. (Withdrawn) A method of treating neoplasia comprising directly injecting into a tumor bed of a patient the nucleic acid molecule encoding a chimeric TNFa polypeptide according to claim 2 wherein the chimeric TNFa polypeptide is expressed in the tumor bed.
52. (Withdrawn) A nucleic acid molecule encoding a chimeric TNFa ligand polypeptide, comprising a first polynucleotide encoding a Domain III fragment of a tumor necrosis factor ligand other than TNFa, wherein the encoded fragment is a homolog of a cleavage site of native TNFa, and a second polynucleotide encoding a Domain IV fragment of TNFa protein that binds to a TNFa receptor.
53. (Withdrawn) The nucleic acid molecule of claim 52, further comprising a third polynucleotide that encodes Domain II domain fragment of the other tumor necrosis factor ligand.
54. (Withdrawn) The nucleic acid molecule of claim 52, further comprising a third polynucleotide that encodes Domain II domain fragment of the other tumor necrosis factor ligand and a fourth polynucleotide that encodes a Domain I domain fragment of the other tumor necrosis factor ligand.
55. (Withdrawn) The nucleic acid molecule of claim 52, wherein the first polynucleotide further encodes a Domain IV domain fragment of the other tumor necrosis factor ligand.
56. (Withdrawn) The nucleic acid molecule of claim 52, wherein the other tumor necrosis factor ligand is selected from the group consisting of CD154, CD70, Fas ligand, NGF, CD30, TNF β , 4-1BBL and TRAIL.
57. (Withdrawn) The nucleic acid molecule of claim 52, comprising a polynucleotide sequence selected from the group consisting of SEQ.ID. NO. 2, SEQ.ID. NO. 3 and SEQ. ID. NO. 4.
58. (Withdrawn) The nucleic acid molecule of claim 52, encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ. ID. NO. 6, SEQ.ID. NO. 7 and SEQ.ID. NO. 8.
59. (Withdrawn) An expression vector containing the nucleic acid molecule of claim 52.
60. (Withdrawn) A host cell containing the nucleic acid molecule of claim 52:

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61. (Withdrawn) A genetic construct containing the nucleic acid molecule of claim 52.
62. (Withdrawn) A chimeric TNFa ligand polypeptide, comprising a Domain III fragment of a tumor necrosis factor ligand other than TNFa, wherein the fragment is a homolog of a cleavage site of native TNFa, and a Domain IV fragment of TNFa protein that binds to a TNFa receptor.
63. (Withdrawn) A method for inducing apoptosis of a cell expressing a TNFa receptor, comprising introducing into the cell an encoding a chimeric TNFa polypeptide according to claim 52 wherein the chimeric TNFa polypeptide is expressed on the surface of the cell.
64. (Withdrawn) A method for inducing activation of an immune system cell, comprising introducing into the cell a nucleic acid molecule encoding a chimeric TNFa polypeptide according to claim 52 wherein the chimeric TNFa polypeptide is expressed on the surface of the cell.
65. (Withdrawn) A method for treating neoplasia in a patient comprising introducing into a neoplastic cell a nucleic acid molecule encoding a chimeric TNFa polypeptide according to claim 52 wherein the chimeric TNFa polypeptide is expressed on the surface of the cell.
66. (Withdrawn) The method of claim 65 further comprising: obtaining the neoplastic cell from a human patient; infusing the neoplastic cell back into the patient after having introduced into the cells the nucleic acid molecule encoding the chimeric TNFa polypeptide.
67. (Withdrawn) A method of treating neoplasia comprising directly injecting into a tumor bed of a patient the nucleic acid molecule encoding a chimeric TNFa according to claim 52 wherein the chimeric TNFa polypeptide is expressed in the tumor bed.